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Bacterioplankton dynamics along the gradient from highly eutrophic Pearl River Estuary to oligotrophic northern South China Sea in wet season: Implication for anthropogenic inputs

Weihua Zhou [a,*], Aimin Long [a], Tao Jiang [b], Shaoyong Chen [a,c], Liangmin Huang [a], Hui Huang [a], Chuanghua Cai [a], Yan Yan [a]

[a] Key Laboratory of Marine Bio-resources Sustainable Utilization (LMB), Key Laboratory of Tropical Marine Environmental Dynamics (LED) and Tropical Marine Biological Research Station in Hainan, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China
[b] Key Laboratory of Aquatic Eutrophication and Control of Harmful Algal Blooms of Guangdong Higher Education Institutes, Jinan University, Guangzhou 510632, China
[c] Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China

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ABSTRACT

Bacterioplankton abundance (BA) and biomass (BB) from the eutrophic Pearl River Estuary (PRE) to the oligotrophic northern South China Sea (NSCS) were studied in the wet season. BA was significantly higher ($p < 0.05$) in PRE ($12.51 \pm 3.52 \times 10^8$ cells L^{-1}), than in the continental shelf neritic province (CSNP, $4.95 \pm 2.21 \times 10^8$ cells L^{-1}) and in the deep oceanic province (OP, $3.16 \pm 1.56 \times 10^8$ cells L^{-1}). Nutrient-replete PRE waters (DIN > 100 μM and PO_4 > 1 μM) resulted in high chl *a* and BB, whereas nutrient-depleted offshore waters (DIN <5 μM and PO_4 < 0.5 μM) had low biomass. Temperature (>26 °C) was not the controlling factor of BA. BB was significantly correlated with chl *a* biomass both in PRE and NSCS. The bacteria to phytoplankton biomass (BB/PB) ratio increased clearly along the gradient from near-shore PRE (0.15) to offshore CSNP (0.93) and deep OP (2.75), indicating the important role of small cells in the open ocean compared to estuarine and coastal zones.

1. Introduction

Bacterioplankton (<2 μm), the bacterial component of the plankton in the aquatic ecosystems, play a central role in the microbial food chain (Azam et al., 1983). On the one hand, bacteria may be important competitors with phytoplankton for inorganic nutrients (Bratbak and Thingstad, 1985). On the other hand, bacteria take up dissolved organic matter (DOM) released by both living and dead phytoplankton and incorporate 10–50% of the carbon fixed by phytoplankton photosynthesis (Fuhrman and Azam, 1982). Bacterial carbon is transferred to larger zooplankton with higher trophic levels via protozoan grazing (Sanders et al., 1989). In most oligotrophic oceans (low chlorophyll *a*), bacterial carbon biomass may even exceed phytoplankton carbon biomass (Fuhrman et al., 1989).

The South China Sea (SCS) is one of the largest marginal seas, which is located in the tropical–subtropical rim of the western North Pacific Ocean (see Fig. 1), encompassing an area from Singapore to Taiwan Strait of around 3.5×10^6 km^2, with an average depth of about 1212 m (Liu et al., 2002; He et al., 2009). Major rivers that flow into SCS include the Pearl, Min, Jiulong, Red, Mekong, Rajang, Pahang, and Pasig Rivers (http://en.wikipedia.org/wiki/South_China_Sea). The northern shelf of SCS receives the outflow of the second largest river (i.e. Pearl River or Zhujiang River) in China. The Pearl River delivers 3.5×10^{11} m^3 yr^{-1} of fresh water with a sediment load of 8.5×10^7 tons yr^{-1} into SCS via three sub-estuaries of Pearl River Estuary (PRE), Lingding Yang, Modao Men, Huangmao Hai (Harrison et al., 2008), with 20% of the discharge occurring during the dry season in October to March and 80% during the wet season in April to September (Yin et al., 2001; Zhao, 1990). Four main outlets, i.e. Hu Men, Jiao Men, Hongqi Men and Heng Men, enter into upper estuary (Fig. 1B). In wet season, the southwest monsoon winds produce an anti-cyclonic circulation in SCS and the SCS warm current flows northwards (Su, 2004; Harrison et al., 2008).

Since economic liberalization was adopted by the Chinese government in the late 1970s, rapid urbanization and industrialization has taken place in the Pearl River Delta (PRD), one of the leading economic regions and major manufacturing centers of China, during the past three decades. It had a population approximately of 60 million people in 2008, and increasing (http://en.wikipedia.org/wiki/Pearl_River_Delta). The PRD is notoriously polluted with sewage and industrial waste treatment facilities failing to keep pace with the growth in population and industry

* Corresponding author. Tel.: +86 20 89023225; fax: +86 20 84451672.
E-mail address: whzhou@scsio.ac.cn (W. Zhou).

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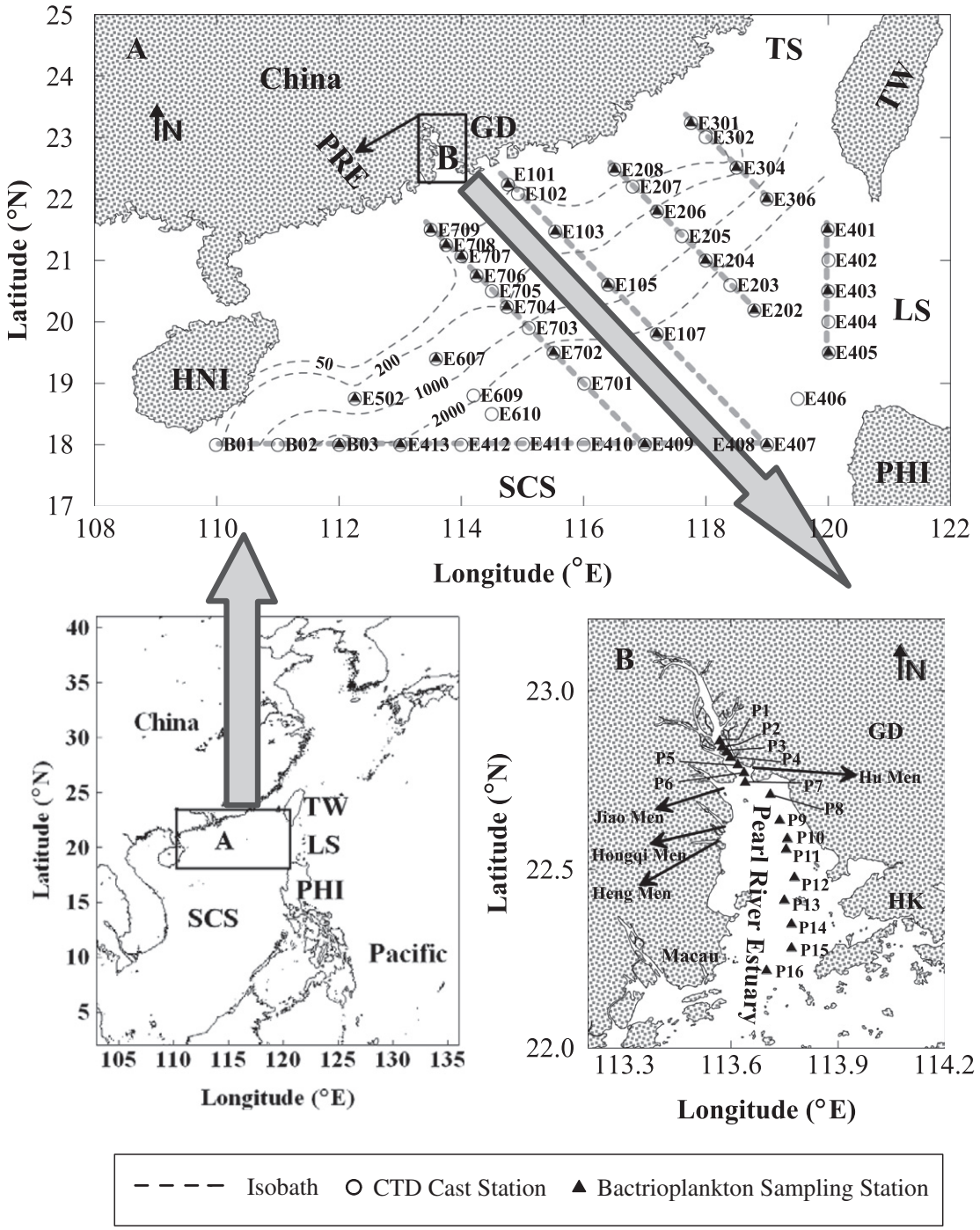

Fig. 1. Sampling stations in northern South China Sea (A) and Pearl River Estuary (B), China. GD: Guangdong; TW: Taiwan; TS: Taiwan Strait; LS: Luzon Strait; PHI: Philippines; SCS: South China Sea; PRE: Pearl River Estuary; HNI: Hainan Island; HK: Hong Kong; thin dashed lines: 50, 200, 1000 and 2000 m depth contours; thick dashed lines: 6 transects.

in the area (Dai et al., 2006; Harrison et al., 2008). The Environment Protection Department of Guangdong Province issued that the total volume of wastewater discharge of 2008 (677 million tons) has almost doubled since 1995 (http://www.gdepb.gov.cn/zwxx/tjsj/index.html). Today, eutrophication is a major environmental issue in PRE and adjacent coastal waters of the northern SCS (Harrison et al., 2008).

Eutrophic environments support a higher standing stock (biomass) of bacteria and phytoplankton than oligotrophic systems (Weinbauer et al., 1993). A marine system, along the salinity and nutrient gradient from nutrient-replete PRE waters to nutrient-limited coastal continental shelf waters and oligotrophic open sea oceanic waters of northern SCS (NSCS), was an ideal model ecosystem for testing this ecological hypothesis. The SCS is oligotrophic with high sea surface temperature (SST), low nutrients (Wu et al., 2003; He et al., 2009), low phytoplankton biomass (chlorophyll *a*) and primary productivity (Liu et al., 2002; Ning et al., 2005). However, few data are available on the spatial distribution of bacterioplankton in such a complicated system along the salinity and nutrient gradient from upper PRE to NSCS. The objectives of present study were to investigate the spatial distribution of bacteria abundance and biomass from eutrophic PRE to oligotrophic NSCS and it's relationships with physical circumstances, nutrients and phytoplankton biomass in wet season.

2. Materials and methods

2.1. Study site and sampling stations

Sampling was conducted in wet season in the subtropical Pearl River Estuary (July 6–13, 2008) and the subtropical–tropical northern South China Sea (September 5–23, 2005), between 18–23° N and 110–120° E (Fig. 1).

The water depth of NSCS stations increased from ~30 m (Stns E301, E302 and E709) in continental shelf to >4000 m (Stns E405, E406 and E407) in southeastern deep basin near Luzon Strait (Fig. 1A). Six transects (45 stations) were designed during the NSCS Open Cruise including four transects running perpendicular to the coastline, covering stations from near-shore and continental shelf neritic province (CSNP, <200 m deep) to deep open ocean or oceanic province (OP, >200 m deep), one transect along the 120° E in Luzon Strait and one transect along 18° N from Hainan Island to Philippines (Fig. 1A).

The water depth of PRE stations ranged from 4 to 14 m, with an average of 8.8 m. One transect with 16 stations were designed to collect samples along the head of PRE near Hu Men to the estuarine coastal plume near Hong Kong waters (Fig. 1B).

2.2. Sampling and measurements

Water samples were collected from surface (0.5 m) and bottom in PRE, surface (0.5 m), 25 m, 50 m and bottom in the CSNP and surface (0.5 m), 25 m, 50 m, 200 m in the OP waters. Profile sampling of one deep station (Stn E409, 3945 m deep) were conducted from surface to 3900 m depth, i.e. surface (0.5 m), 50 m, 100 m, 200 m, 300 m, 500 m, 800 m, 1000 m, 1500 m, 2000 m, 3000 m and 3900 m.

Water temperature and salinity were measured with SBE 911 plus CTD (Sea-Bird Electronics, Inc., Washington, USA). Unfortunately, no salinity data were available during the PRE cruise due to the sensor breakdown. Water samples for bacterioplankton and other parameters were collected with 2.5 L Niskin bottles on a CTD rosette. Water samples (100–500 ml according to phytoplankton biomass) for chlorophyll *a* (chl *a*) were passed through Whatman GF/F filters (nominal pore size of 0.7 μm). The filtrates were collected for analyses of dissolved nutrients (Dissolved Inorganic Nitrogen, DIN, orthophosphate and silicate). Chl *a* and nutrients (DIN = NO_3 + NO_2 + NH_4, PO_4 and SiO_4) were measured by 10-AU Turner Designs Fluorometer and Skalar San Plus Autoanalyzer, respectively, after the Joint Global Ocean Flux Study (JGOFS) protocols (Knap et al., 1996).

Bacterioplankton samples were preserved with 3.7% formaldehyde (v/v, final concentration) in the sterilized sealed tubes, and then stored at 4 °C (dark) in refrigerator until laboratory analysis. Bacteria were counted by an epifluorescence microscope (OLYMPUS BX41, Japan) with a 100 W high pressure mercury burner for epifluorescence illumination, after staining with DAPI (4′, 6-diamidino-2-phenylindole dihydrochloride, Sigma) at least for 5 min in the dark (Velji and Albright, 1986; Zheng and Cai, 1993). Attached bacteria were dislodged by dispersing them in a suspension medium using a combination of chemical treatment (10 mM sequestering and deflocculating agent, tetrasodium pyrophosphate) and ultrasound (300 r min^{-1}) for 40 s in an ice-cold circumstance. The detailed procedures were described in Zhou et al. (2009).

Bacterial biomass (BB) was calculated from the bacterial abundance with a relatively constant cell to carbon conversion factor of 20 fg C $cell^{-1}$ for natural free-living bacterial assemblages (Lee and Fuhrman, 1987). Phytoplankton biomass (PB, mg C m^{-3}), was determined from a conversion factor of 50 mg C per mg chl *a* (Krempin and Sullivan, 1981; Zhou et al., 2009).

2.3. Quality control and data statistical analysis

The bottles and glassware for sampling and analysis were acid cleaned by soaking in 10% HCl solution overnight. The chemicals and solutions used in this study were analytical reagent. Results are expressed as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) and standard multiple regressions were performed using SPSS 13.0 statistical analysis software. Differences were considered statistically significant when $p < 0.05$. Contours plotting of different layers and transects were obtained with the SURFER 8.0 program (Golden Software).

3. Results

3.1. Physical environment in PRE and NSCS during the wet season

The annual average flow rate approaches approximately 10,000 m^3 s^{-1} for Pearl River, with 70–80% occurring in wet season from April to September (Zhao, 1990; Harrison et al., 2008). The salinity varied from 1 to >33 (salinity value of 33 is taken to represent the oceanic water). Light fresh water dominated the surface waters of PRE and the front extends across the estuary during the wet season, with the salinity varied from 1 to ~20 (Fig. 2a). The salt oceanic water intruded along the axis of the estuary and the bottom salinity increased across the estuary from the west to east (Fig. 2b; Dong et al., 2004). In July 6–13, 2008, the temperature ranged from 26.4 °C (bottom) to 28.2 °C (surface) in PRE waters, with a difference <2 °C between the surface and bottom waters, and averaged 27.5 ± 0.2 °C in the surface and 27.1 ± 0.2 °C in the bottom.

In NSCS, surface salinity ranged from 29.7 (Stn E708) to 34.1 (Stn E411). On average, the surface salinity in continental shelf neritic province (CSNP) and oceanic province (OP) were 32.9 ± 1.1

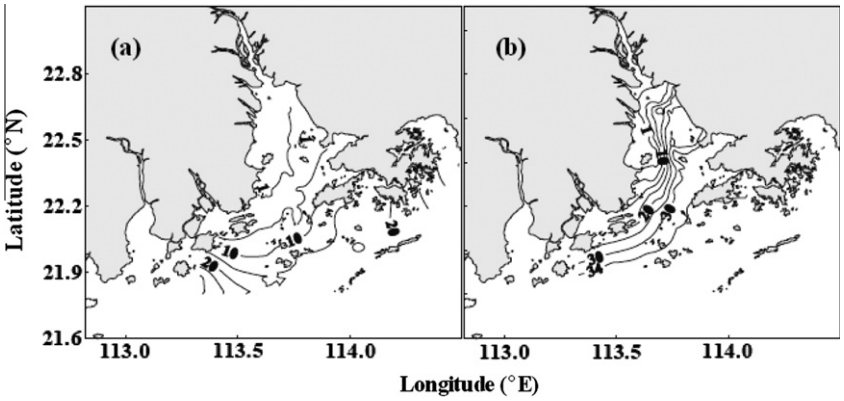

Fig. 2. Salinity distribution contours at surface (a) and bottom (b) waters in Pearl River Estuary in wet season (after Dong et al., 2004).

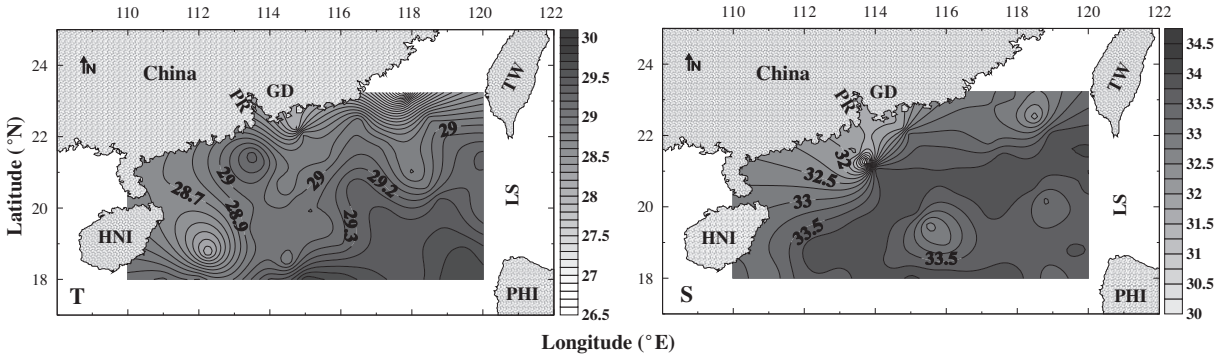

Fig. 3. Horizontal distribution contours of temperature (T, °C) and salinity (S) at surface layer in northern South China Sea.

(ranged from 29.7 to 33.9) and 33.7 ± 0.4 (ranged from 32.6 to 34.1), respectively. The surface temperature in CSNP and OP were 28.7 ± 0.7 °C (ranged from 26.8 °C to 29.4 °C) and 29.2 ± 0.3 °C (ranged from 28.1 to 29.8 °C), respectively.

An estuarine coastal plume appeared near the Pearl River mouth with relative lower salinity than OP in NSCS (Fig. 3). A warm water mass with high salinity was evidently observed between Taiwan and Philippines, which was probably the surface water of the warm Kuroshio Current from Luzon Strait intruding into northern SCS began in late summer (Shaw, 1991) and flowing westward along the continental margin of south China (Fig. 3, also observed by He et al. (2009)).

3.2. Nutrients and phytoplankton biomass

Surface DIN decreased from >100 μM in the upper estuary to ~80 μM in the lower estuary (averaged 123.0 ± 31.2 μM), ranged from 3 to 7 μM in CSNP (averaged 4.9 ± 2.0 μM), and 1–2 μM in OP (averaged 1.5 ± 0.8 μM). PO_4 and SiO_4 also showed strong gradients from estuary to deep ocean. Surface PO_4 ranged from 0.8 to 1.7 μM in PRE (averaged 1.1 ± 0.3 μM), 0.4 to 0.7 μM in CSNP (averaged 0.5 ± 0.2 μM), and <0.5 μM in deep OP waters (averaged 0.37 ± 0.15 μM). Surface SiO_4 ranged from 25 to 120 μM in PRE (averaged 69.1 ± 25.2 μM), 3–10 μM in CSNP (averaged 7.1 ± 3.5 μM) and <4 μM in deep OP waters (averaged 4.0 ± 0.2 μM) (Fig. 4). Since most DIN/PO_4 ratios were >100:1 in the river estuary and <16:1 in CSNP and OP, phytoplankton growth potentially became P-limitation in PRE and N-limitation in continental shelf and OP waters (Fig. 4).

During the wet season, high river freshwater outflow dilutes phytoplankton biomass in the estuary. Chl *a* biomass were mostly lower than 5 mg m^{-3} due to high turbid flow, except for Stns P1–P4 (ranged from 5 to 8 mg m^{-3}) in the upper stream near Hu Men (Figs. 1 and 4). Surface chl *a* concentrations were lower than 0.1 mg m^{-3} in OP, ~0.2 mg m^{-3} in CSNP, and ranged from 2 to 8 mg m^{-3} in PRE. Averaged chl *a* biomass in the water column were 4.61 ± 1.89 mg m^{-3} in PRE, 0.29 ± 0.21 mg m^{-3} in CSNP, and 0.10 ± 0.11 mg m^{-3} in deep open ocean (Table 1).

3.3. Horizontal distribution of bacterioplankton

Generally, bacterial abundance (BA) decreased seaward from PRE to open OP waters. On average, BA was about 3-fold and 4-fold higher in the estuarine waters than that in CSNP and OP, respectively (Table 2). The bacterial distribution along the gradient from PRE to OP was similar to that of chl *a* (Fig. 4). Average surface BA was 14.32 ± 3.44 × 10^8 cells L^{-1} in PRE, 5.17 ± 2.09 × 10^8 cells L^{-1} in CSNP, and 3.82 ± 1.01 × 10^8 cells L^{-1} in OP.

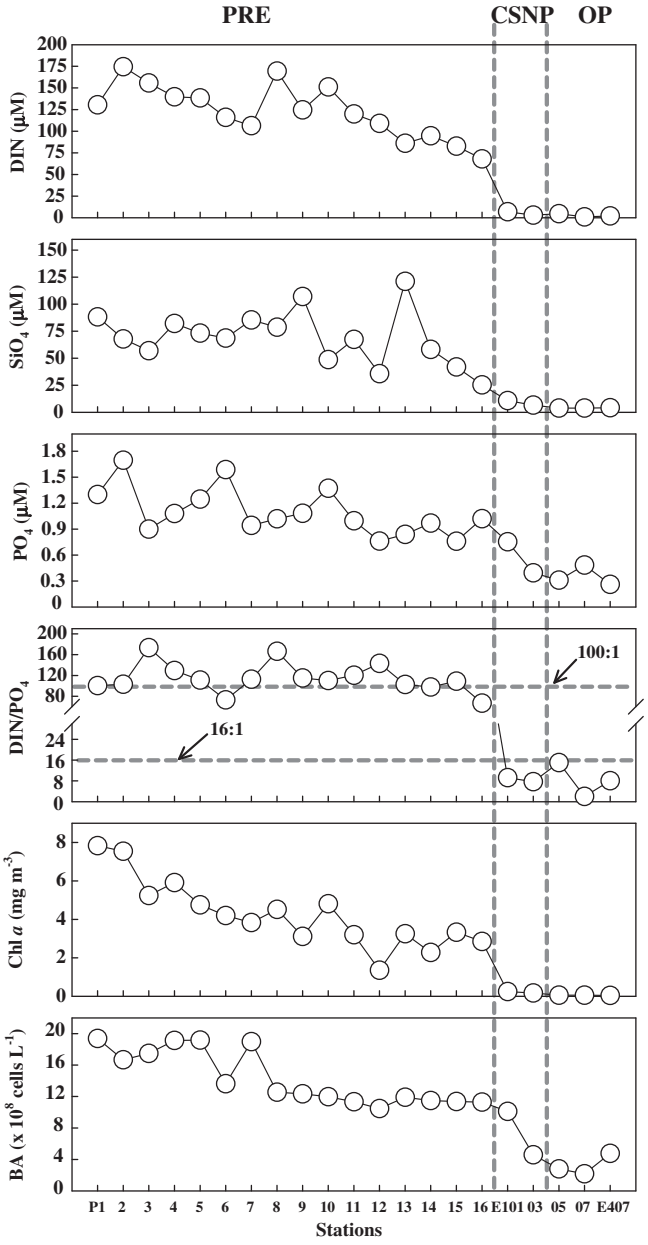

Fig. 4. Nutrients (DIN, SiO_4 and PO_4), DIN/PO_4 ratio, bacterial abundance (BA) and chl *a* of a transect from Pearl River Estuary (PRE, Stns P1 to P16) to continental shelf neritic province (CSNP, Stns E101 and E103) and oceanic province (OP, Stns E105, E107 and E407) at surface waters in northern South China Sea.

Table 1
Chl *a* (mg m^{-3}) in Pearl River Estuary (PRE), continental shelf neritic province (CSNP, <200 m deep) and oceanic province (OP, >200 m deep).

Layer	PRE	CSNP	OP
Surface	4.25 (1.76)	0.22 ± 0.14	0.06 ± 0.01
Average	4.61 ± 1.89	0.29 ± 0.21	0.10 ± 0.11

Table 2
Bacterial abundances (BA, $\times 10^8$ cells L^{-1}) in PRE, CSNP (<200 m) and OP (>200 m).

Layer	PRE	CSNP	OP
Surface	14.32 ± 3.44	5.17 ± 2.09	3.82 ± 1.01
25 m	–	6.87 ± 3.75	4.32 ± 1.53
50 m	–	4.61 ± 0.85	4.19 ± 1.35
200 m	–	–	1.62 ± 0.50
Bottom	10.70 ± 2.61	4.18 ± 1.33	–
Average	12.51 ± 3.52	4.95 ± 2.21	3.16 ± 1.56

Spatial distribution of BA showed clearly variability with decreasing from upper to lower estuary in PRE (Fig. 5). Surface BA was much higher in west coast of estuary than that of east coast, indicating the influence of the fresh water discharge. Highest BA occurred at the head of estuary near Hu Men both at the surface and bottom waters. At the bottom layer, BA was much higher in east coast near Hong Kong and Shenzhen than west coast, which was probably induced by vast sewage discharge from these metropolises of Pearl River Delta (Fig. 5).

In NSCS, surface BA decreased from the coast to the open ocean, with the high values ($\sim 10 \times 10^8$ cells L^{-1}) appeared near the eutrophic Pearl River estuarine plume and low abundances ($<3 \times 10^8$ cells L^{-1}) beyond the 200 m contour and continental slope between Taiwan and Hainan Island (Fig. 6). At 25 m depth, highest values were detected both at estuarine plume and Taiwan Strait probably due to the influence of river discharge and Taiwan Warm Current, respectively. Relatively high abundances were observed in Taiwan Strait and the southeast in OP at both 50 m and 200 m depth. In the entire water column, the lowest values occurred at the same zone in the west of Luzon Strait, located at the continental slope between CSNP and OP (Fig. 6). In most of 200 m depth of OP, bacterial abundances were less than 2×10^8 cells L^{-1}.

3.4. Vertical distribution of bacterioplankton

The gradient of bacterial abundance was apparently larger in the stations near the estuary of transects E1 and E7 (perpendicular to the coastline, located on both sides of the estuary) than deep ocean, indicating a clear estuarine influence (Fig. 7). At transects E2 and E3, bacterial abundance was much higher at near shore stations, and gradually reduced seaward (Fig. 7). The lowest bacterial abundances ($<3 \times 10^8$ cells L^{-1}) were observed in the entire water column at Stns E105 and E107 (Figs. 4 and 7).

Bacterial abundance was higher at the surface than the bottom waters in PRE stations (Fig. 5 and Table 2). It might be caused by the oligotrophic oceanic waters strongly intruding at the bottom layer during the wet season (Yin et al., 2001). Among water column of most stations, the highest BA occurred in subsurface layer in CSNP and in OP (25 m or 50 m), and then surface layer followed (Fig. 7 and Table 2). BA was mostly lower than 2×10^8 cells L^{-1} at the waters deeper than 200 m (Figs. 7 and 8). At 200–1000 m of a deep station E409, BA was ranged between 1 and 1.5×10^8 cells L^{-1}, and changed little ($\sim 0.5 \times 10^8$ cells L^{-1}) when lower than 1500 m depth (Fig. 8).

4. Discussion

Bacterial abundance is mainly controlled by bottom-up regulation in an oligotrophic ocean due to low nutrients and dissolved organic carbon (DOC) concentrations, while controlling by top-down regulation often occurs in eutrophic waters (Cole et al., 1988). In contrast to some other studies, observations in the present study demonstrate that bacterial abundance does follow nutrient-related phytoplankton biomass levels, and supports the view that bacterioplankton are important members in the Microbial Food Loop of various marine ecosystems, playing a critical role in carbon and energy cycles (Azam et al., 1983). The distribution pattern of bacterial abundance indicates that bacteria respond to anthropogenic nutrients input from land runoff, especially from vast Pearl River outflow in the NSCS marine ecosystems.

4.1. Physical processes effect on bacterial abundance

Physical processes, such as river discharge, estuarine plume, stratification, tidal front, and upwelling/downwelling, play an important role in governing the spatial distribution of bacterial abundance (Li et al., 2007; He et al., 2009).

During the wet season, the estuary of Pearl River is highly stratified with a very low salinity (<1 at the head of the estuary) at surface layer extending seawards and a deep water salinity front (oceanic water) intruding along the axis of the estuary (Harrison et al., 2008). Surface bacteria were significantly higher than that of bottom in PRE (Fig. 5).

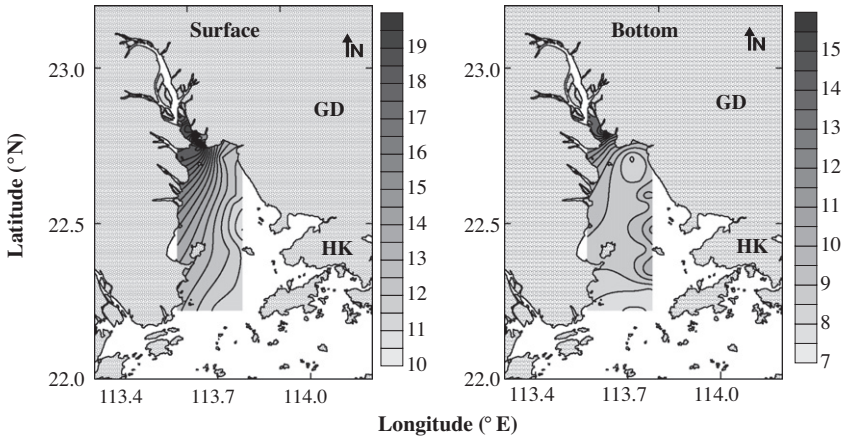

Fig. 5. Horizontal distribution contours of bacterial abundance (BA, $\times 10^8$ cells L^{-1}) at surface and bottom waters in Pearl River Estuary.

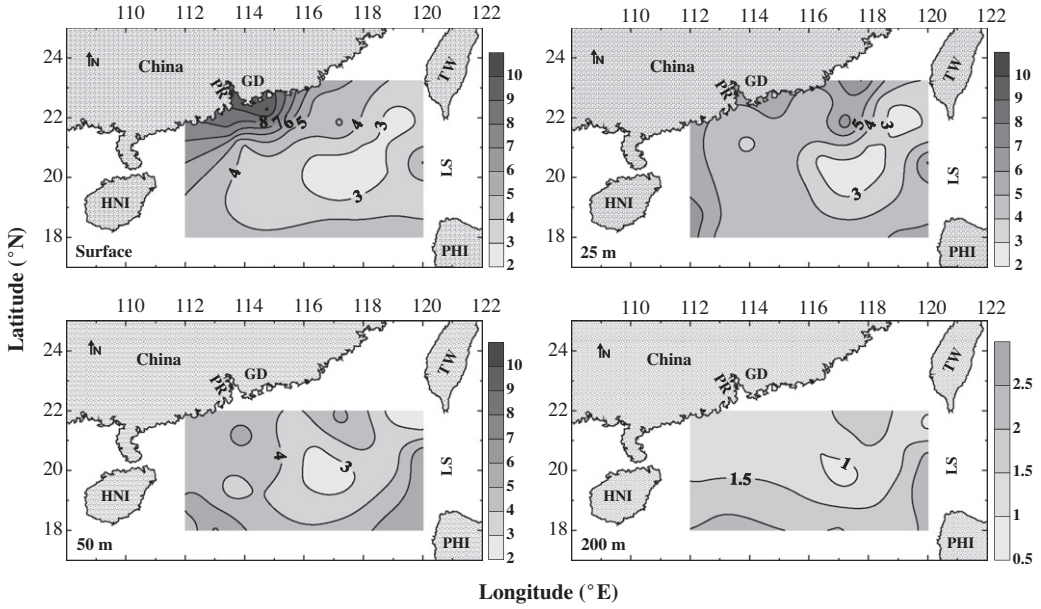

Fig. 6. Horizontal distribution contours of bacterioplankton abundance (BA, $\times 10^8$ cells L^{-1}) at surface, 25 m, 50 m and 200 m depth in northern South China Sea.

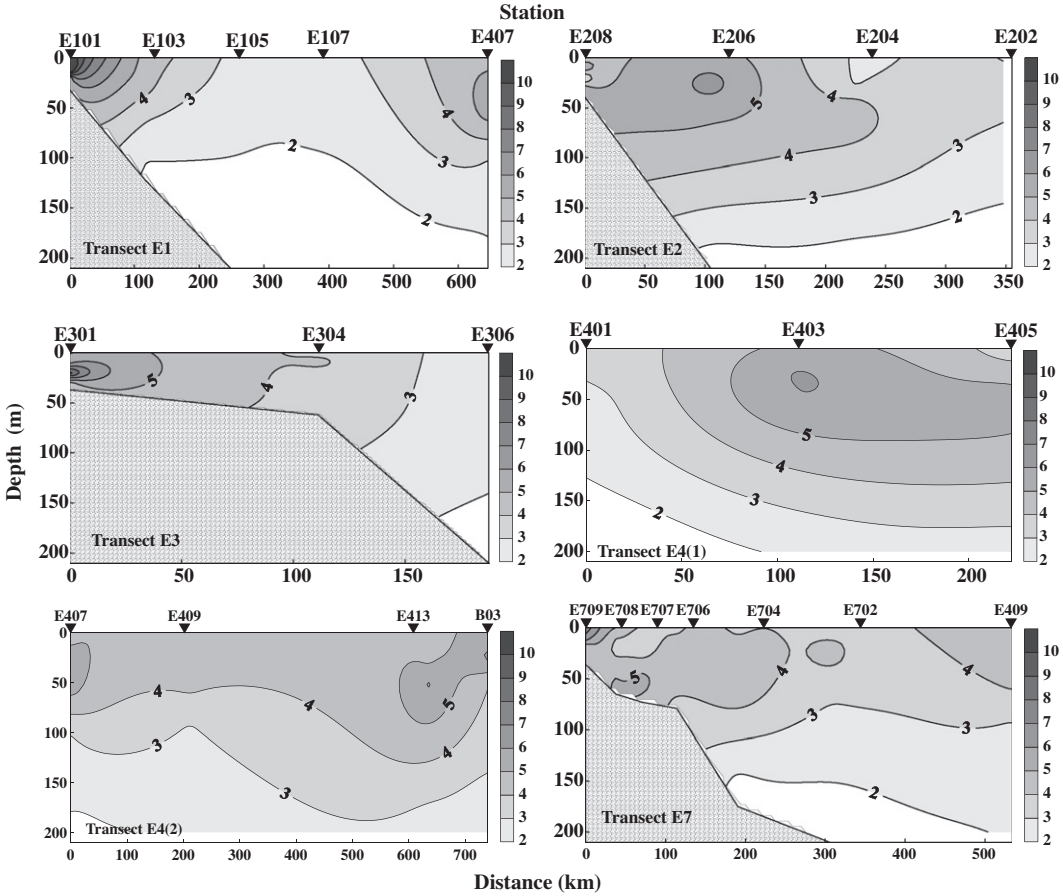

Fig. 7. Depth contours of bacterial abundance (BA, $\times 10^8$ cells L^{-1}) along the transect E1 (E101–E407), E2 (E202–208), E3 (E301–306), E4 (1) (E401–E405), E4 (2) (B03–E407) and E7 (E709–E409) in northern South China Sea. Arrows indicate the positions of the stations where vertical profiles were taken.

The spatial distribution of bacterial abundance at surface layer exhibits strong horizontal gradient as salinity from nearshore to open sea mainly due to terrestrial runoff and the Pearl River outflow (Figs. 2, 3, 5 and 6). Although no salinity data were available in PRE during sampling, bacteria were still negatively correlated with salinity in NSCS ($r = -0.4727$, $n = 26$, $p = 0.0147$).

According to Shiah and Ducklow (1994a,b), temperature played a major role in regulative functions in Chesapeake Bay and became the main regulating factor of bacterioplankton growth than

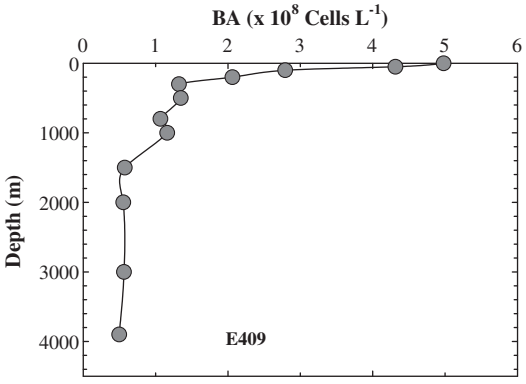

Fig. 8. Vertical distribution of bacterial abundance (BA, $\times 10^8$ cells L^{-1}) at Stn E409 (3945 m deep) in northern South China Sea.

Table 3
Surface bacterial biomass (BB) to phytoplankton biomass (PB) ratio (BB/PB) in PRE, CSNP (<200 m) and OP (>200 m).

Item	PRE	CSNP	OP
Average	0.15 ± 0.05	0.93 ± 0.44	2.75 ± 0.85
Range	0.09–0.31	0.28–1.67	1.56–4.60

nutrients, except for summer when temperature >20 °C. The incubation results at a low temperature level showed that temperature was the most important factor influencing bacterioplankton growth at temperate waters (Vrede, 2005). Since PRE and NSCS locate at the region from subtropical to tropical, the SST ranged from 26.8 to ~30 °C during the cruise time. There was no evident correlation between temperature and bacterial abundance both in PRE (temperature almost keep constant during sampling period, with a mean of 27.5 ± 0.2 °C) and NSCS ($r = 0.3111$, $n = 26$, $p = 0.1218$). Temperature was not the controlling factor of the distribution pattern of phytoplankton and bacterial biomass in PRE and the adjacent NSCS in wet season.

4.2. Anthropogenic input of nutrients effect on bacterial abundance

The Pearl River discharge is an important nutrient source for the ecosystems both in PRE and continental shelf of NSCS (Su, 2004). Be consistent with Harrison et al. (2008), the high anthropogenic inputs of nitrogen from land sewage runoff and the Pearl River outflow (DIN > 100 μM) yield a high DIN/PO_4 ratio (>100 N:1P) in PRE waters, which was about seven times higher than the Redfield optimal phytoplankton growth ratio of 16 N:1P (Fig. 4).

During the wet season, abundant low salinity waters from the Pearl River outflow occupied the coastal waters beyond PRE (Fig. 3), resulting in high nutrients (He et al., 2009). Strong nutrients gradient were exhibited from PRE to coastal waters and offshore deep waters (Fig. 4). Summer bioassay experiment demonstrated that low salinity regions in PRE were potentially P-limited, while the outer estuary was N-limited (Zhang et al., 1999; Yin et al., 2001, 2004; Harrison et al., 2008).

Nutrients (DIN, PO_4 and SiO_4) had no correlations with bacterial abundance in Pearl River estuarine waters ($p > 0.05$), probably due to excessive nutrients for phytoplankton and bacteria growth. But considered as a whole system from PRE to NSCS, the low salinity water mass with higher nutrient concentrations resulted in higher phytoplankton biomass (chl *a*), bacterial abundance, similar to the results of He et al. (2009). Bacterial abundance was significantly correlated with DIN ($r = 0.9054$, $n = 42$, $p < 0.0001$), PO_4 ($r = 0.3693$, $n = 42$, $p = 0.0161$), SiO_4 ($r = 0.8689$, $n = 42$, $p < 0.0001$) and DIN/PO_4 ratio ($r = 0.8656$, $n = 42$, $p < 0.0001$). This mainly resulted from the anthropogenic nutrients input from land runoff and the Pearl River outflow. As a result, low salinity PRE and coastal waters with higher nutrient concentrations resulted in higher phytoplankton and bacterioplankton biomass, whereas higher salinity oligotrophic offshore water mass at deep basin had lower chl *a* and bacterial biomass.

4.3. Correlation between bacterial biomass (BB) and phytoplankton biomass (PB)

The balance between phytoplankton photosynthesis and bacterial heterotrophic respiration is a major function of the Microbial Food Loop in regulating CO_2 as a sink or source (Legendre and Rivkin, 2002). Several researchers have suggested that coastal and shelf ecosystems with high productivity might be a net CO_2 source due to high bacterial production, although this issue is still being debated (Smith and Hollibaugh, 1993; Del Giorgio et al., 1997).

Dissolved organic matter (DOM) originating from phytoplankton in the euphotic zone is an important substrate for bacterial growth in most marine systems (Azam et al., 1993), especially in systems with reduced external influence as in oligotrophic open ocean case (Conan et al., 1999). Bacterial abundances were more significantly correlated with phytoplankton biomass (chl *a*) in NSCS ($r = 0.7398$, $n = 26$, $p < 0.0001$) than in PRE ($r = 0.7369$, $n = 32$, $p = 0.0011$), which probably showed that phytoplankton released DOM played a more important role for bacterial growth in NSCS than in PRE waters.

Generally, the ratio of bacterial biomass (BB) to phytoplankton biomass (PB), BB/PB, reflects the importance of bacterioplankton in the marine biogeochemical cycling (Cherrier et al., 1996). Average BB/PB ratio showed significantly regional difference along the estuary-offshore gradient, increasing from PRE (0.15) to offshore CSNP (0.93) and deep OP (2.75) (Table 3), indicating the smaller cells played much more important role in open ocean than coastal zones.

Bacterial biomass tends to be much lower than phytoplanktonic biomass in eutrophic waters (Gasol et al., 1997). Our results in PRE is consistent with the conclusion of Ducklow and Carlson (1992), who affirmed that the BB/PB ratio of near-shore is generally <0.2, except for Stn P12 (with a value of 0.31). Average BB/PB ratio in PRE (0.15) was almost the same as that in Sanya Bay (~18° N), Hainan Island, at the same season in 2005 (Zhou et al., 2009). The BB/PB ratio in CSNP (0.93, September) was slightly lower than that of the Yellow Sea (0.97, October), and BB were close to PB in both sea regions (Zhao et al., 2003).

5. Conclusions

Similar to chl *a* biomass and nutrients distribution pattern, bacteria abundance decreased seaward along the salinity gradient from PRE to offshore CSNP and deep OP waters. Temperature, at a high level during the cruise (>26 °C), was not the controlling factor of regulating the distribution pattern of phytoplankton and bacterial biomass in PRE and the adjacent NSCS in wet season. Whereas anthropogenic nutrients input from Pearl River outflow and land runoff was the main governing factor. Compared with large-sized phytoplankton, smaller cells, such as bacterioplankton (<2 μm), played a much more important role in offshore deep ocean than estuarine and coastal waters.

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References

Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A., Thingstad, F., 1983. The ecological role of water-column microbes in the sea. Marine Ecology Progress Series 10, 257–263.

Azam, F., Smith, D.C., Steward, G.F., Hagström, Å., 1993. Bacterial organic matter coupling and its significance for oceanic carbon cycling. Microbial Ecology 28, 167–179.

Bratbak, G., Thingstad, T.F., 1985. Phytoplankton–bacteria interactions: an apparent paradox? Analysis of a model system with both competition and commensalism. Marine Ecology Progress Series 25, 23–30.

Cole, J.J., Findlay, S., Pace, M.L., 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. Marine Ecology Progress Series 43, 1–10.

Conan, P., Turley, C., Stutt, E., Pujo-Pay, M., Van Wambeke, F., 1999. Relationship between phytoplankton efficiency and the proportion of bacterial production to primary production in the Mediterranean Sea. Aquatic Microbial Ecology 17, 131–144.

Cherrier, J., Bauer, J.E., Druffel, E.R.M., 1996. Utilization and turnover of labile dissolved organic matter by bacterial heterotrophs in eastern North Pacific surface waters. Marine Ecology Progress Series 139, 267–279.

Dai, M.H., Guo, X.H., Zhai, W.D., Yuan, L.Y., Wang, B.W., Wang, L.F., Cai, P.H., Tang, T.T., Cai, W.J., 2006. Oxygen depletion in the upper reach of the Pearl River Estuary during a very drought winter. Marine Chemistry 102, 159–169.

Del Giorgio, P.A., Cole, J.J., Cimbleris, A., 1997. Respiration rates in bacteria exceed plankton production in unproductive aquatic systems. Nature 385, 148–151.

Dong, L., Su, J., Wong, L.A., Cao, Z., Chen, J.C., 2004. Seasonal variation and dynamics of the Pearl River plume. Continental Shelf Research 24, 1761–1777.

Ducklow, H.W., Carlson, G.A., 1992. Oceanic bacterial production. In: Marshall, K.C. (Ed.), Advances in Microbial Ecology. Plenum, New York, pp. 113–181.

Fuhrman, J.A., Sleeter, T.D., Carlson, C.A., Proctor, L.M., 1989. Dominance of bacterial biomass in the Sargasso Sea and its ecological implications. Marine Ecology Progress Series 57, 207–217.

Fuhrman, J.A., Azam, F., 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Marine Biology 66, 109–120.

Gasol, J.M., del Giorgio, P.A., Duarte, C.M., 1997. Biomass distribution in marine planktonin communities. Limnology and Oceanography 42, 1353–1363.

Harrison, P.J., Yin, K., Lee, J.H.W., Gan, J., Liu, H., 2008. Physical–biological coupling in the Pearl River Estuary. Continental Shelf Research 28, 1405–1415.

He, L., Yin, K., Yuan, X., Li, D., Zhang, D., Harrison, P.J., 2009. Spatial distribution of viruses, bacteria and chlorophyll in the northern South China Sea. Aquatic Microbial Ecology 54, 153–162.

Knap, A., Michaels, A., Close, A., Ducklow, H., Dickson, A., 1996. Protocols for the Joint Global Ocean Flux Study (JGOFS) Core Measurements. JGOFS Report No. 19. Reprint of the IOC Manuals and Guides No. 29. UNESCO, Paris.

Krempin, D.W., Sullivan, C.W., 1981. The seasonal abundance, vertical distribution, and relative microbial biomass of chroococcoid cyanobacteria at a station in southern California coastal waters. Canadian Journal of Microbiology 27, 1341–1344.

Lee, S., Fuhrman, J.A., 1987. Relationships between biovolume and biomass of naturally derived marine bacterioplankton. Applied and Environmental Microbiology 53, 1298–1303.

Legendre, L., Rivkin, R.B., 2002. Fluxes of carbon in the upper ocean: regulation by food-web control nodes. Marine Ecology Progress Series 242, 95–109.

Li, H.B., Xiao, T., Lv, R.H., Ding, T., Lin, Y.A., 2007. Impact of tidal front on the distribution of bacterioplankton in the southern Yellow Sea, China. Journal of Marine Systems 67, 263–271.

Liu, K.K., Chao, S.Y., Shaw, P.T., Gong, G.C., Chen, C.C., Tang, T.Y., 2002. Monsoon-forced chlorophyll distribution and primary production in the South China Sea: observations and a numerical study. Deep-Sea Research Part I 49, 1387–1412.

Ning, X., Li, W.K.W., Cai, Y., 2005. Standing stock and community structure of photosynthetic picoplankton in the northern South China Sea. Acta Oceanologica Sinica 24, 57–76.

Sanders, R.W., Porter, K.G., Bennett, S.J., De Biase, A.E., 1989. Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. Limnology and Oceanography 34, 673–687.

Shaw, P.T., 1991. The seasonal variation of the intrusion of the Philippine Sea water into the South China Sea. Journal of Geophysical Research 96 (C1), 821–827.

Shiah, F.K., Ducklow, H.W., 1994a. Temperature regulation of heterotrophic bacterioplankton abundance, production, and specific growth rate in Chesapeake Bay. Limnology and Oceanography 39, 1243–1258.

Shiah, F.K., Ducklow, H.W., 1994b. Temperature and substrate regulation of bacterial abundance, production and specific growth rate in Chesapeake Bay, USA. Marine Ecology Progress Series 103, 297–308.

Smith, S.V., Hollibaugh, J.T., 1993. Coastal metabolism and the oceanic organic carbon balance. Reviews of Geophysics 31, 75–89.

Su, J., 2004. Overview of the South China Sea circulation and its influence on the coastal physical oceanography outside the Pearl River Estuary. Continental Shelf Research 24, 1745–1760.

Velji, M.I., Albright, L.J., 1986. Microscopic enumeration of attached marine bacteria of seawater, marine sediment, fecal matter, and kelp blade samples following pyrophosphate and ultrasound treatments. Canadian Journal of Microbiology 32, 121–126.

Vrede, K., 2005. Nutrient and temperature limitation of bacterioplankton growth in temperate lakes. Microbial Ecology 49, 245–256.

Weinbauer, M.G., Fuks, D., Peduzzi, P., 1993. Distribution of viruses and dissolved DNA along a coastal trophic gradient in the northern Adriatic Sea. Applied and Environmental Microbiology 59, 4074–4082.

Wu, J., Chung, S.W., Wen, L.S., Liu, K.K., Chen, Y.L.L., Chen, H.Y., Karl, D.M., 2003. Dissolved inorganic phosphorus, dissolved iron, and *Trichodesmium* in the oligotrophic South China Sea. Global Biogeochemical Cycles 17, 1008. doi:10.1029/2002GB001924.

Yin, K., Qian, P.Y., Wu, M.C.S., Chen, J.C., Huang, L., Song, X., Jian, W., 2001. Shift from P to N limitation of phytoplankton biomass across the Pearl River estuarine plume during summer. Marine Ecology Progress Series 221, 17–28.

Yin, K., Song, X., Sun, J., Wu, M.C.S., 2004. Potential P limitation leads to excess N in the Pearl River estuarine coastal plume. Continental Shelf Research 24, 1895–1907.

Zhang, J., Yu, Z.G., Wang, J.T., Ren, J.L., Chen, H.T., Xiong, H., Dong, L.X., Xu, W.Y., 1999. The subtropical Zhujiang (Pearl River) estuary: nutrient, trance species and their relationship to photosynthesis. Estuarine, Coastal and Shelf Science 49, 385–400.

Zhao, H.T, 1990. Evolution of the Pearl River Estuary. China Ocean Press, Beijing, 358 pp. (in Chinese).

Zhao, S.J., Xiao, T., Yue, H.D., 2003. The distribution of heterotrophic bacteria and some related factors in the East China and Yellow Seas during fall. Oceanologia et Limnologia Sinica 34, 295–305 (in Chinese with English abstract).

Zheng, T.L., Cai, L.Z., 1993. The role of marine bacteria in upwelling ecosystem. Acta Oceanologica Sinica 12, 261–267.

Zhou, W.H., Li, T., Cai, C.H., Huang, L.M., Wang, H.K., Xu, J.R., Dong, J.D., Zhang, S., 2009. Spatial and temporal dynamics of phytoplankton and bacterioplankton biomass in Sanya Bay, northern South China Sea. Journal of Environmental Sciences 21, 595–603.